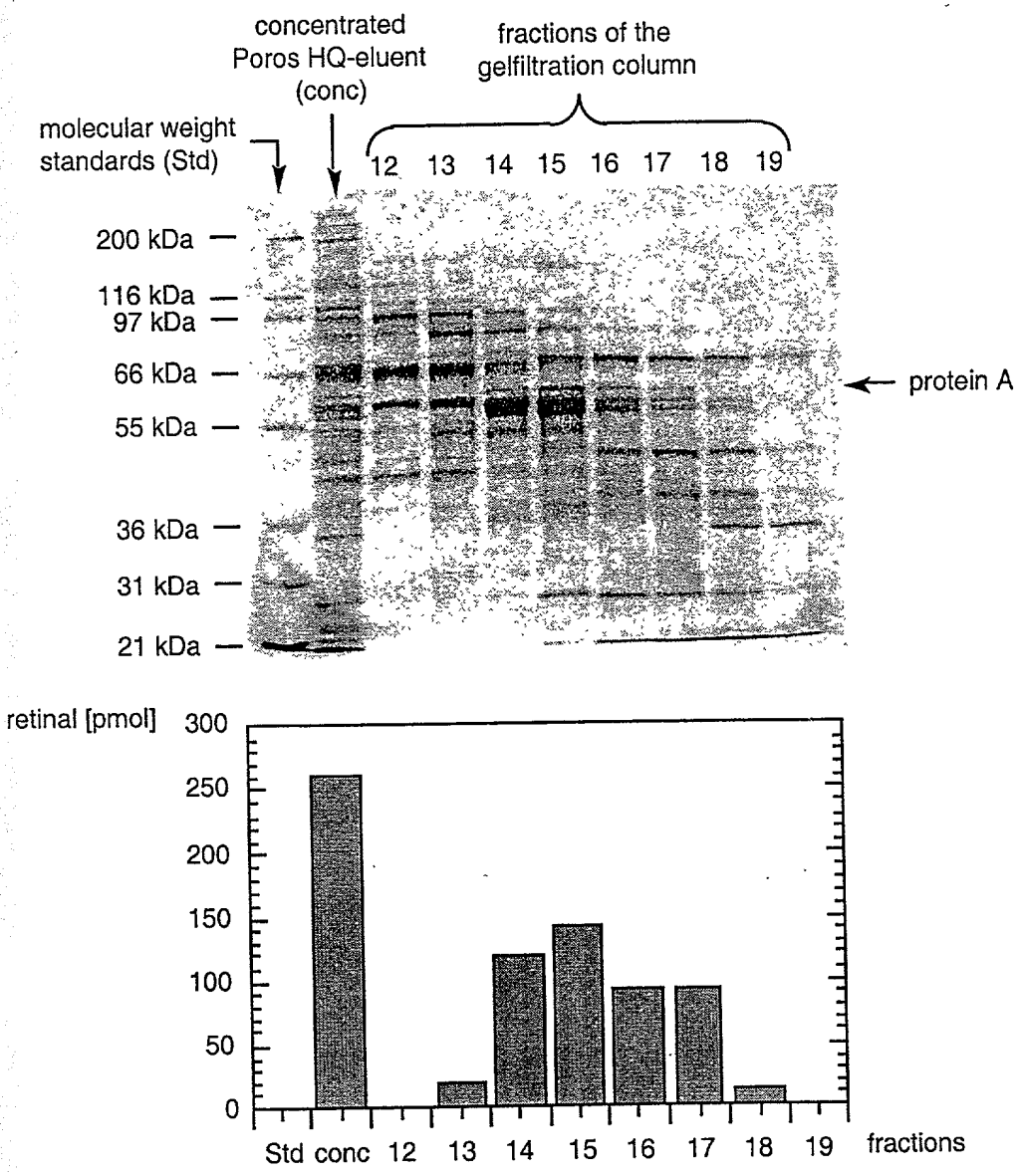
Figure 1



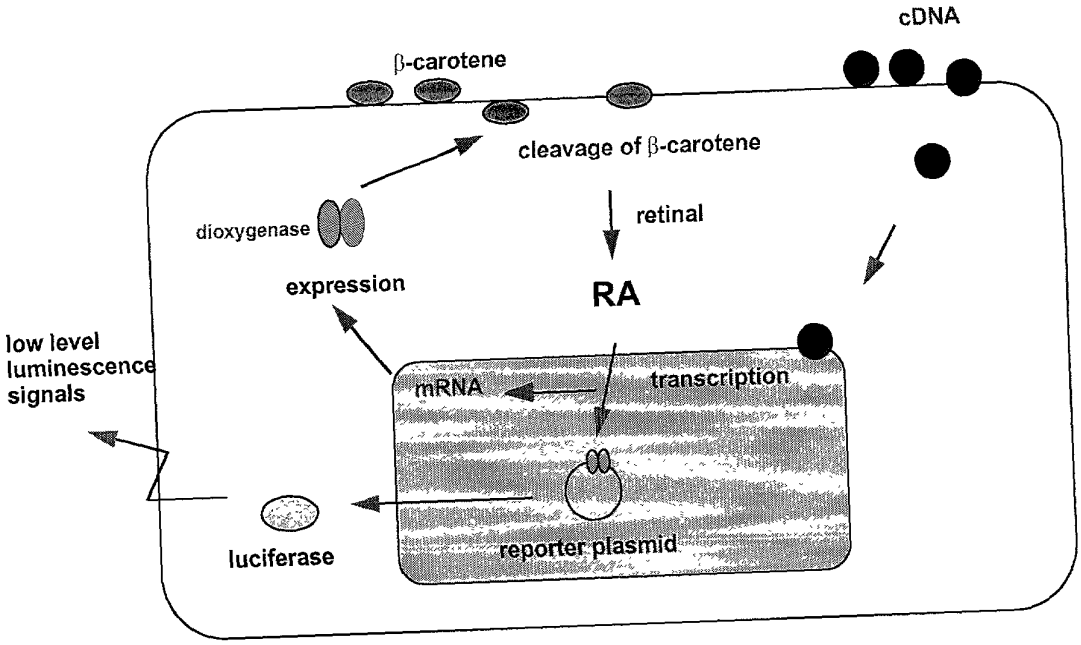

cDNA
β-carotene
cleavage of β-carotene
dioxygenase
retinal
expression
RA
low level
luminescence
signals
mRNA
transcription
luciferase
reporter plasmid

Figure 2

Figure 3 Seq. ID
No. 2

1 CGGATCCACT AGTAACGGCC GCCAGTGTGG TGGAATCCAT
CCTTCTATGT

51 AACAGGAAAG AGCTGTTCTT AGCCCAGAGA GGAGGGCACC
GTACGCCTGC

101 AGGAGCAGCT GGGTAGAGGA CACAGGAGAG CGATGGAGAC
AATATTTAAC

151 AGAAACAAAG AAGAGCATCC AGAGCCCATA AAAGCTGAGG
TGCAAGGTCA

201 GTTGCCCACT TGGTTGCAAG GGGTACTTCT CCGAAATGGC
CCAGGGATGC

251 ACACAATAGG GGACACTAAA TACAACCACT GGTTTGATGG
CTTGGCTCTG

301 CTGCACAGCT TCACGTTTAA AAATGGTGAA GTTTACTACA
GAAGTAAGTA

351 CCTCCGAAGT GACACATACA ACTGCAATAT AGAAGCAAAC
CGAATCGTGG

401 TGTCTGAGTT TGGAACCATG GCTTATCCGG ATCCATGCAA
AAACATATTT

451 GCCAAGGCAT TCTCATACTT ATCTCACACC ATTCCTGAGT
TCACGGACAA

501 CTGCCTGATC AACATTATGA AAACTGGGGA TGATTATTAT
GCTACCAGTG

551 AGACTAACTT CATCAGAAAA ATTGATCCAC AGACTCTGGA
GACACTAGAT

601 AAGGTAGACT ACAGCAAATA TGTAGCTGTA AACTTGGCAA
CTTCTCACCC

651 ACACTATGAC AGTGCTGGAA ATATTCTCAA CATGGGTACT
TCAATTGTTG

701 ATAAAGGGAG AACAAAATAT GTTCTCTTTA AGATCCCTTC
CTCTGTACCA

751 GAAAAAGAAA AGAAGAAATC TTGTTTTAAA CACCTGGAAG
TAGTATGCTC

801 CATCCCTTCT CGCTCCCTGC TCCAACCAAG CTACTACCAC
AGCTTTGGAA

851 TCACAGAAAA TTATATTGTG TTCATAGAGC AGCCATTTAA
ACTGGATATT

901 GTCAAACTGG CAACTGCCTA CATCCGAGGT GTGAACTGGG CTTCCTGCCT

951 TTCCTTTCAT AAGGAGGATA AGACGTGGTT TCACTTTGTA GACAGAAAGA

1001 CGAAAAAAGA AGTATCCACC AAGTTTTACA CTGATGCTTT GGTGCTTTAT

1051 CACCACATAA ATGCTTACGA AGAAGATGGC CACGTTGTTT TTGATATCGT

1101 TGCCTACAGA GACAATAGCT TGTACGATAT GTTTTACTTA AAAAAACTGG

1151 ACAAAGACTT TGAAGTGAAC AACAAGCTTA CCTCCATCCC AACCTGCAAG

1201 CGCTTTGTTG TGCCTCTGCA GTATGACAAG GATGCAGAAG TAGGTTCTAA

1251 TTTAGTCAAA CTTCCAACTT CCGCAACTGC TGTAAAAGAA AAAGATGGCA

1301 GCATCTATTG TCAACCTGAA ATATTATGTG AAGGGATAGA ACTGCCTCGT

1351 GTCAACTATG ACTACAATGG CAAAAAATAC AAGTATGTCT ATGCAACAGA

1401 AGTCCAGTGG AGCCCAGTTC CTACAAAGAT TGCAAAACTG AATGTCCAAA

1451 CAAAGGAAGT ACTGCACTGG GGAGAAGACC ACTGCTGGCC CTCAGAGCCC

1501 ATCTTTGTTC CCAGCCCCGA TGCAAGAGAA GAGGATGAAG GTGTTGTTTT

1551 GACCTGTGTT GTGGTGTCTG AGCCAAATAA AGCACCCTTC CTACTCATCT

1601 TGGATGCTAA AACATTCAAA GAATTGGGCC GAGCCACAGT TAACGTAGAA

1651 ATGCATCTGG ACCTGCATGG GATGTTTATA CCACAGAATG ATTTGGGGGC

1701 TGAGACGGAA TAAAACGCTA TTGATCCGAC TACACAAACT GAGACAACTT

1751 TCTACTGAAC ATGAGTTAAT ATCCCTTTTA CCATTCAAGA ACAACCATAT

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2251 CAACTTTCTA ATTTACAACC GAAACAAACA AGCAAACAGC ACTTGCTTTG

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2601 TGTAGGTGAA AATGCATTTA CTGATGAAAG TTGAATCGTT AATGAGGGAG

2651 AAAACTGGGT ATCCATCCAT CCAACTATGT TAGGTGTTCA CCTGGTCTGT

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2751 ATGGTTTCTG CTACTCACTG TATTTTGCAG GAGAGAAACA AAATGAAATC

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AAAAAAAAAA

3101 AAAAAAAAAA A

Figure 4
No. 1

Seq. ID

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101 PCKNIFAKAF SYLSHTIPEF TDNCLINIMK TGDDYYATSE TNFIRKIDPQ

151 TLETLDKVDY SKYVAVNLAT SHPHYDSAGN ILNMGTSIVD KGRTKYVLFK

201 IPSSVPEKEK KKSCFKHLEV VCSIPSRSLL QPSYYHSFGI TENYIVFIEQ

251 PFKLDIVKLA TAYIRGVNWA SCLSFHKEDK TWFHFVDRKT KKEVSTKFYT

301 DALVLYHHIN AYEEDGHVVF DIVAYRDNSL YDMFYLKKLD KDFEVNNKLT

351 SIPTCKRFVV PLQYDKDAEV GSNLVKLPTS ATAVKEKDGS IYCQPEILCE

401 GIELPRVNYD YNGKKYKYVY ATEVQWSPVP TKIAKLNVQT KEVLHWGEDH

451 CWPSEPIFVP SPDAREEDEG VVLTCVVVSE PNKAPFLLIL DAKTFKELGR

501 ATVNVEMHLD LHGMFIPQND LGAETE

Figure 5

Seq ID No. 4 and Seq ID No. 5

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57
      ||   |: | | |.:| || | |||   |||:  :|   : | ||| |||
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69
               .         .         .         .         .
   58 HSFTFKNGEVYYRSKYLRSDTYNCNIEANRIVVSEFG..TMAYPDPCKNI
105
      | | || | | |  :::|.| |   .   |||:.|||  | |:|||||||
   70 HKFDFKEGHVTYHRRFIRTDAYVRAMTEKRIVITEFGFTTCAFPDPCKNI
119
               .         .         .         .         .
  106 FAKAFSYLSHTIPEFTDNCLINIMKTGDDYYATSETNFIRKIDPQTLETL
155
      |.: |||      | ||| |:|:   |:|||| .||||| ||.|:||||:
  120 FSRFFSYFRGV..EVTDNALVNVYPVGEDYYACTETNFITKINPETLETI
167
               .         .         .         .         .
  156 ..DKVDYSKYVAVNLATSHPHYDSAGNILNMGTSIVDKGRTKYVLFKIPS
203
         .||   ||.|| ||.||| :. | : |.|          | : |||
  168 FTKQVDLCNYVSVNGATAHPHIENDGTVYNIGNCFGKNFSIAYNIVKIPP
217
               .         .         .         .         .
  204 SVPEKEKKKSCFKHLEVVCSIPSRSLLQPSYYHSFGITENYIVFIEQPFK
253
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  218 LQADKEDPISKFTS.EIVVQFPCSDRFKPSYVHSFGLTPNYIVFVETPVK
266
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  254 LDIVKLATAY.IRGVNWASCL.SFHKEDK.TWFHFVDRKTKKEVSTKFYT
300
      :.: |  ..: : | |:  |  || |     | |  |:| || .. |: |
  267 INLFKFLSSWSLWGANYMDCFESFTNETMGVWLHIADKKRKKYLNNKYRT
316
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  301 DALVLYHHINAYEEDGHVVFDIVAYRDNSL...YDMFYLKKLDKDFE...
344
          |:|||| ||:.| .: |:  ::       |   ||  | ...|
  317 SPFNLFHHINTYEDNGFLIVDLCCWKGFEFVYNYFTLYLANLRENWEEVK
366
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  345 VNNKLTSIPTCKRFVVPLQYDKDAEVGSNLVKLP.TSATAV..KEKDGSI
391
       | :    |  :|:|.||  || |: | ||| || |.|||:   :.  .|
  367 KNARKAPQPEVRRYVLPLNIDK.ADTGKNLVTLPNTTATAILCSDEFTTI
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436
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464
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    465 NVKTKETWFTVWQEPDSYPSEPIFVSHPDALEEDDGVVLSVVVSPGAGQK
514

                  .              .             .
    485 P.FLLILDAKTFKELGRA..TVNVEMHLDLHGMF 515
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    515 PAYLLILNAKDLSEVARAEFTVEINIPVTFHGLF 548
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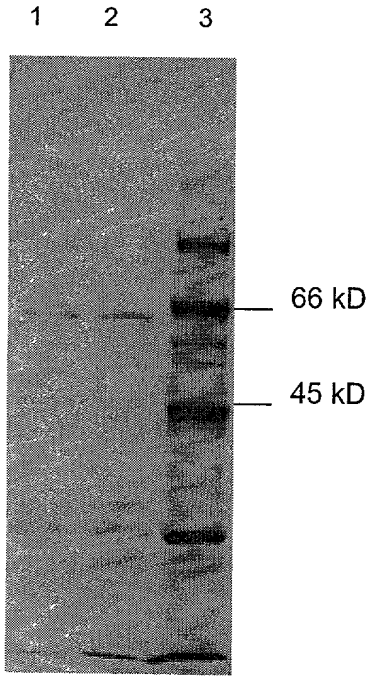

Fig. 6 shows a 10% polyacrylamide gel with E.coli expressed β,β-carotene 15,15'-monooxygenase after affinity tag purification; lane 1 and lane 2: 2 fractions from the Co^{2+}-chelate column showing the main band at 60 kD; lane 3: low range molecular weight marker (Bio Rad).

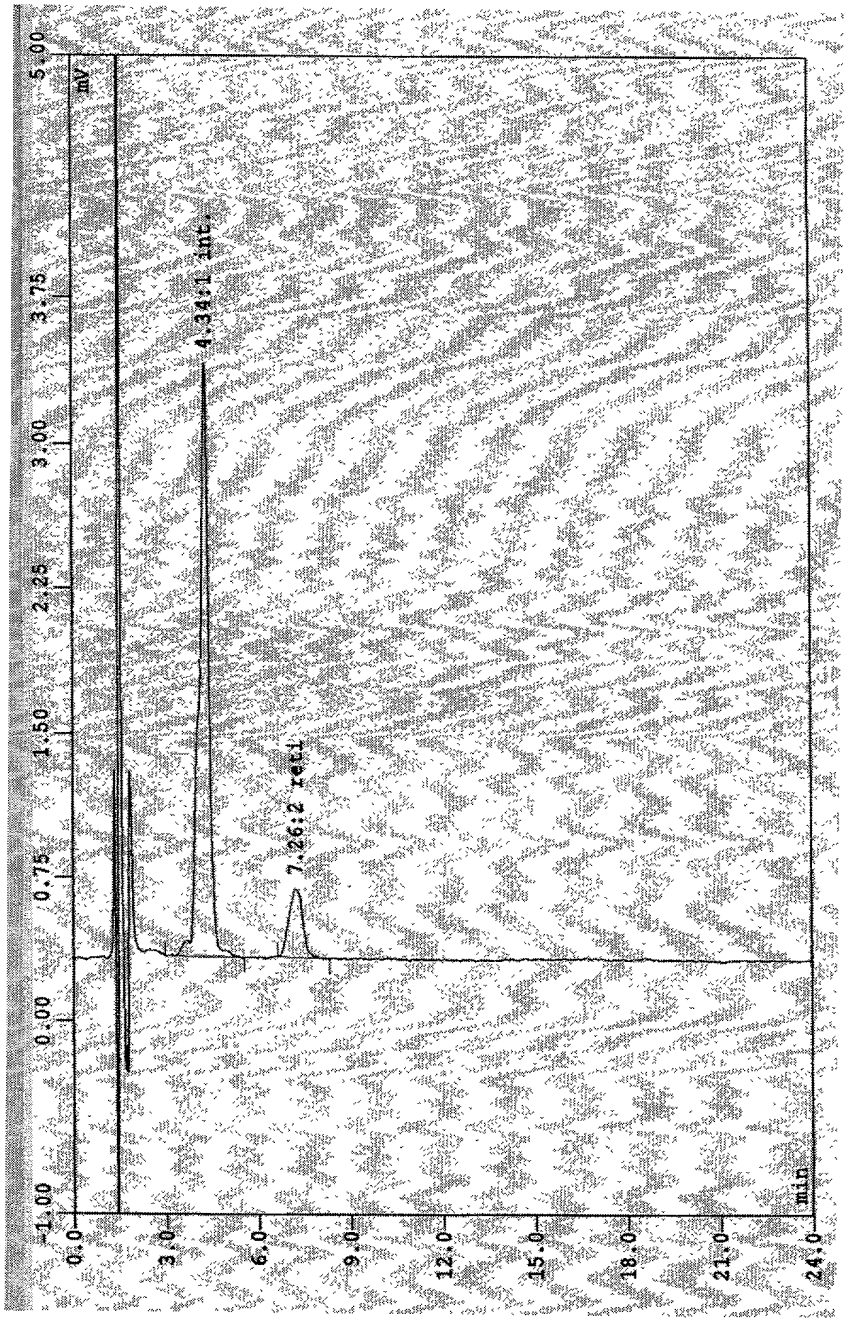

Fig. 7 shows an HPLC profile of the reaction mixture at the end of an actvity assay for the β,β-carotene 15,15'-monooxygenase following the procedure in example 1. The first peak in the chromatogram represents the internal standard, while the second peak corresponds to retinal as the only product formed during the central cleavage with β-carotene as substrate.

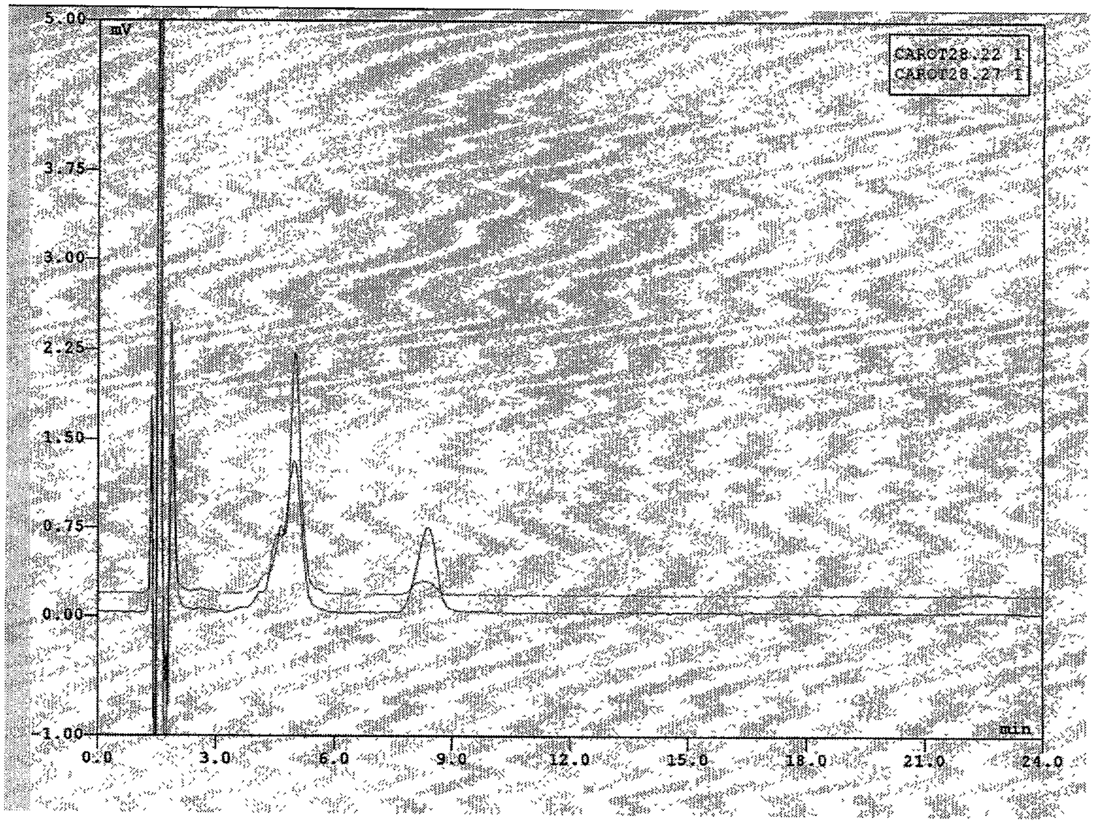

Fig. 8 confirms that the product peak in Fig. 7 is indeed retinal. A sample which was positive in the activity assay (green (upper) chromatogram) was spiked with retinal and analysed in second HPLC run (red (lower) chromatogram). The chromatograms of the two runs were then overlayed.